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# Ecological priming of artificial aquaculture structures: Kelp farms as an example

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## ABSTRACT

*The continued development of the aquaculture industry is contributing to the proliferation of artificial structures in the marine environment. Observations of seaweed farms (infrastructure and biomass) suggest they act as a habitat for associated species. Seaweed farms differ from other forms of artificial infrastructure as the material deployed already has marine organisms (i.e. culture species) growing on it. This 'priming' of ropes with juvenile sporophytes may affect future development of communities by facilitating colonizing species or suppressing competitors. We call this process 'ecological priming': the provision of a biological platform that influences the successional development of specific communities. The communities that developed on ropes primed with* Alaria esculenta *individuals were compared to unprimed ropes to assess the ecological priming effect, at a commercial kelp farm in southwest Ireland. Species richness increased over two cultivation seasons and species composition was consistent between years, with distinct communities developing on primed and unprimed treatments. Timing of species occurrence on primed ropes was predictable with no predictable pattern occurring on unprimed ropes. Multivariate tests indicated distinct communities between treatments, with suppression of other algal species and potential facilitation of some species that have a particular association with* A. esculenta *on primed ropes. Communities from primed holdfasts contained a lower diversity of algal species compared to unprimed communities. Cultivated kelp holdfasts represent a habitat for distinct assemblages that reflect ecological priming of the substratum.*



## KEYWORDS

Artificial structures*;* Aquaculture; *Alaria esculenta;* Macroalgae; Holdfasts; Epibionts; Predictability; Facilitation; Kelp farm;

## INTRODUCTION

Over the last few decades there has been rapid proliferation of artificial structures in the marine environment (Firth *et al.*, 2016a) to enable the exploitation of the ocean's energy and food resources. Novel structures include oil and gas platforms, marine renewable energy installations and aquaculture facilities (Chapman & Underwood, 2011; Firth & Hawkins, 2011). The term 'ocean sprawl' has been used to describe this expansion of coastal and marine infrastructure (Duarte *et al.,* 2012*;* Firth *et al.*, 2016a, 2016b) and this is gaining recognition as one of the biggest threats to marine ecosystems (Airoldi & Beck, 2007; Firth *et al.*, 2013; Dafforn *et al.*, 2015; Bishop *et al.,* 2017; Heery *et al.,* 2017).

Specifically, the aquaculture industry has grown dramatically over the last 50 years to an all-time high of 101 million tonnes live weight in 2014 (FAO, 2016). Within the aquaculture sector, the cultivation of aquatic plants (dominated by marine macroalgae), is also expanding rapidly: by almost 8 % per year over the past decade (FAO, 2016). Over 33 % of the 27.3 million tonnes of global annual aquatic plant production came from just two kelp species (*Laminaria japonica* Areschoug, (1851) and *Undaria pinnatifida* (Harvey) Suringar (1873)) (FAO, 2016). Kelp species are cultivated to produce biomass to supply the many traditional (e.g. food) and expanding uses (e.g. biofuels) of kelp (Guiry, 1989; Walls *et al.*, 2016). Observations of the artificial infrastructure associated with seaweed farms and the kelp biomass itself suggest that farms provide important ecosystem functions and services such as habitat provision (Park *et al.*, 1990; Peteiro & Freire, 2013; Førde *et al.*, 2016; Walls *et al.*, 2016, 2017), protection from predators, and farms may act as nursery grounds for juvenile fish species, similar to that of wild kelp forests (Smale *et al.*, 2013; Walls *et al.*, 2016). However, cultivated kelps are grown suspended from ropes in the water column whereas wild kelps grow attached to the benthos, and this alteration of environments could modify the provision of these services (Walls *et al.*, 2016). Seaweed farms differ from other forms of artificial infrastructure in that the material placed in the sea already has marine organisms growing on it. This 'priming' of ropes with juvenile sporophytes may affect subsequent development of the fouling community by facilitating colonizing species or suppressing competitors. The intended consequence of seeding ropes with sporophytes is that a thick growth of harvestable kelp biomass develops. We term this process 'ecological priming' and define it as the practice of providing a biological platform that influences the successional development of specific communities. In this study, artificial structures (ropes), seeded with organisms, juvenile kelp (*Alaria esculenta* (Linnaeus) Greville (1830)) sporophytes, will be

64 referred to as 'primed' structures and conversely artificial structures with no seeding will be referred to as
65 'unprimed' structures.
66
67 To date the majority of research on the role of kelp as a habitat has focused on the holdfast structure (Jones,
68 1971; Moore, 1973; Schultze *et al.*, 1990; Smith, 1996; Thiel & Vásquez, 2000; Christie *et al.*, 2003; Blight &
69 Thompson, 2008; Walls *et al.*, 2016; Teagle *et al.*, 2017). This focus on holdfasts is due in part to the relative
70 ease in collecting these discrete sampling units (Walls *et al.*, 2016) and because the holdfast is generally found
71 to host the highest diversity of all kelp structures (such as, kelp stipes and fronds) (Jones, 1972; Thiel &
72 Vásquez, 2000; Norderhaug *et al.*, 2002; Christie *et al.*, 2003; Arroyo *et al.*, 2004). Within an individual
73 holdfast, species richness typically reaches 30 – 70 macrofaunal species (Jones, 1972; Thiel & Vásquez, 2000;
74 Teagle *et al.,* 2017), but in some cases, may reach up to 90 species (Christie *et al.*, 2003). This relatively high
75 biodiversity is thought to reflect the complex physical structure provided by the holdfast, as the branched root-
76 like shape of the holdfast provides a number of holes and crevices as living space (Christie *et al.*, 2003). This
77 interstitial space may represent favourable habitat for colonising fauna, potentially providing protection from
78 predators and during periods of adverse environmental conditions (Norderhaug *et al.*, 2002). The holdfast
79 functions as a sediment trap accumulating detritus, which acts as a food source for many of the organisms
80 inhabiting the structure (Moore, 1972). The holdfast also provides a stable environment which is persistent over
81 seasons and years (Schaal *et al.*, 2009); with the lifespan of the holdfast being the same as that of the kelp
82 individual (Kain, 1963; Christie *et al.*, 2003). This stability contrasts with the seasonally fluctuating habitat
83 experienced by stipe-associated epiphytes (Norderhaug, 2004) and to the temporally renewing frond habitat
84 (Christie *et al.*, 2003, 2007). It must be noted that, depending on cultivation practices, entire kelp individuals
85 including the holdfast can be harvested from the farm thus the lifespan of cultivated holdfasts is only as long as
86 the cultivation period.
87
88 A number of biotic and abiotic factors may influence the assemblages found on both seeded kelp droppers
89 (ropes suspended vertically from seaweed farms which form substrate for cultivated kelps) and on submerged
90 infrastructure like ropes. Where kelps form holdfasts, biotic factors include holdfast age, morphology and
91 habitat volume. Wild holdfasts are perennial; Sheppard *et al.,* (1980) found that species richness increased for
92 three different age classes of *Laminaria hyperborea* (Gunnerus) Foslie (1884) holdfasts. Age is linked to habitat
93 volume, as holdfasts continually grow through the addition of more haptera and more space is enclosed within.

Here, habitat volume is defined as the space available for colonisation by organisms within the holdfast (see Walls *et al.,* (2016)). The volume and structure of these interstitial spaces have been shown to impact the diversity and abundance of associated assemblages (Jones, 1971; Thiel & Vásquez, 2000; Blight & Thompson, 2008; Tuya *et al.*, 2011; Walls *et al.*, 2016). The distinct holdfast morphologies of wild and cultivated *L. digitata* (Hudson) Lamourx (1813) were suggested as the cause of variations in species richness and community composition by Walls *et al.,* (2016). Wild kelps tend to grow a characteristic flat or slightly conical holdfast when attached to rock (Figure 1a in Walls *et al.,* 2016)), whilst cultivated kelps are seeded onto ropes, resulting in a different morphology, formed by intertwined haptera around the rope substratum (Figure 2A and Figure 1b in Walls *et al.,* 2016)). Alterations in abiotic conditions experienced by the holdfast can also cause variations in the assemblages inhabiting the holdfast (Smith, 2000; Walls *et al.*, 2016). Smith (1996) found differences in community structure between holdfasts sampled at different depths (2 m and 6 m), however depth also influences sediment load, structural complexity and water turbulence (Smith, 2000). Changing from a benthic to a suspended substratum can alter both the hydrodynamic environment and sedimentation rates experienced by fauna (Walls *et al.*, 2016). Shifts in other abiotic conditions that influence holdfast assemblages (and by extension, rope-attached assemblages) include hydrodynamic environment (Moore, 1972), sedimentation rates (Schaal *et al.*, 2012), light availability, and temperature (Scarratt, 1961).

In this study, we focus on the assemblage that develops on suspended ropes primed with the cultivated kelp *Alaria esculenta*. Kelp-associated holdfast epibionts were surveyed at different times during the two cultivation periods to track changes in community composition and development. We compared these primed rope communities with communities that developed on unprimed ropes. The comparison of primed and unprimed treatments allows for an estimation of the effect of 'ecological priming'. We tested the hypothesis that a priming effect alters the development of assemblages differently across primed and unprimed treatments. An assessment of an ecological priming effect associated with cultivated kelps is novel and, if present, may have important implications for habitat restoration and enhancement of artificial structures. If the development of primed communities is predictable, this would increase the capacity for planning and management in the seaweed cultivation industry. Also, if cultivated holdfasts are found to have distinct assemblages when compared to unprimed treatments, this suggests they supplement the habitat service provided by artificial structures, such as mooring and anchor ropes, with the farm providing an alternative habitat for associated communities. This study builds on previous work conducted in Walls *et al.,* 2017 on the ecological processes occurring on cultivated kelp

124 farms. However, the data presented here is related to the communities associated with the holdfast material

125 while, Walls *et al.,* 2017 studied the frond fouling communities and their impact on commercial aspects of

126 seaweed farming.

# MATERIALS AND METHODS

## Study site

This study was conducted in southwest Ireland in Ventry Harbour, County Kerry (52° 06' 49.45"N, 10° 21'20.17"W; Figure 1) at the largest operating commercial seaweed farm in Ireland (18 ha site). Ventry Harbour is a moderately sheltered and shallow embayment orientated towards the southeast, approximately 2.5 x 1.5 km (3.75 $km^2$) with a wide mouth opening into Dingle Bay. The seagrass *Zostera marina* Linnaeus (1753) is extensively distributed throughout the sandy seabed, leading to a rocky boulder reef towards the mouth of the bay. Wild kelp populations are found at the mouth of Ventry Harbour and on the northeastern shore of the bay, approximately 250 m- 1 km from the farm site). The licensed seaweed farm is orientated northwest to southeast, and located to the westerly side of Ventry Harbour (Figure 1). The depth underneath the farm is approximately 6 m at the northwestern end before gently sloping to 20 m at the eastern edge of the farm at mean low water spring tide (MLWS). The tidal range in Ventry Harbour is between 0.6 and 4.0 m. Monthly irradiance values, obtained from nearby Valentia weather observatory (51° 56' 23" N, -10° 14' 40" W), ranged from 5,447 – 63,823 J $cm^{-2}$ for 2014; equivalent to approximately 3,356 to 40,364 mmol photons $m^{-2}$ $day^{-1}$ using the approximation suggested by Tett (1990). Sea surface temperature data were obtained from the M3 offshore weather buoy located approximately 56 km southwest of Mizen head (51° 13' 0" N, 10° 33' 0" W), and ranged from 10.1 – 17.6 $^{o}$C for 2014. Although offshore sea temperatures are less extreme than inshore waters, Ventry Harbour is a well-flushed bay so values are broadly representative. The longline set-up and design of the seaweed farm is described in detail in Walls *et al.,* 2017. The farm cultivates the kelps *Alaria esculenta* and *Saccharina latissima* (Linnaeus) Lane, Mayes, Druehl & Saunders (2006) for human consumption, animal feed and use in cosmetic products.

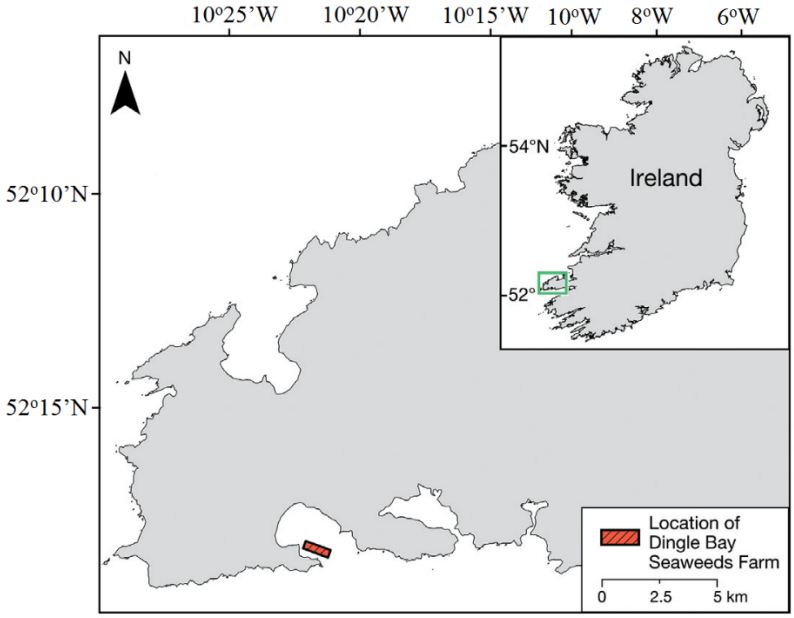


Fig. 1. Location of Dingle Bay Seaweeds farm and sampling site at Ventry Harbour, Co. Kerry, Ireland.

## Experimental set-up

Experimental treatments were based on 1 m polypropylene dropper ropes (10 mm diameter) with two initial set-ups. Treatment 1 "primed droppers" consisted of ropes sprayed directly with juvenile *A. esculenta* sporophytes that had been developed from gametophyte cultures held in the seaweed hatchery at the NUIG Carna Research Station (County Galway), following standard industry protocols. The primed ropes were left to develop under controlled growth conditions (Edwards & Watson, 2011) for between 5 to 12 weeks. Sporophytes were ca. 10 mm at time of deployment. Treatment 2 dropper ropes consisted of clean polypropylene rope and will be referred to as "unprimed droppers" hereafter. Unprimed droppers were submerged in tanks of seawater under the same laboratory conditions as the primed droppers for the same length of time prior to deployment. At deployment, all dropper replicates had a 1 kg concrete weight attached to the end of the rope and were deployed vertically on the longline header rope and spaced 1.5 m apart to mitigate against rubbing and tangling (Walls *et al.*, 2016). Dropper ropes were suspended at a depth of between 1.5 to 2.5 m below the surface of the water, which is a depth range experienced at commercial seaweed farms. Each dropper was randomly assigned to a location on the longline header rope prior to deployment. Primed (n=35) and unprimed (n=35) droppers were deployed on the 18$^{th}$ February 2014 for the 2013/2014 growing season (Year 1); deployment was delayed in Year 1 due to winter storms in early 2014. The experiment was repeated for the 2014/2015 growing season (Year 2), when the primed (n=35) and unprimed (n=35) treatments were deployed on the 15$^{th}$ December 2014.

## Sampling protocol for primed and unprimed droppers

All samples were collected by SCUBA divers. In April, May and June 2014 and 2015 five droppers were randomly chosen and collected from the primed and unprimed treatments using open-ended mesh bags (150 x 55 cm, 0.5 mm mesh size). If a dropper was not uniformly covered in developing sporophytes (i.e. showed evidence of rubbing or entanglement), another dropper replicate was selected. The mesh bag was carefully slipped up over the dropper and tightly secured at top and bottom (just above the weight) using cable ties, enclosing the entire 1 m dropper and kelp biomass. The focus for the current study was to compare the assemblages associated most closely with the dropper rope. There are potential issues of habitat extent when comparing the assemblages of entire kelp sporophytes with organisms attached to unprimed rope (where large kelp blades did not develop in the experimental time period). We therefore compared the near-rope assemblages of the holdfast with those on unprimed droppers.

All samples were transferred back to nearby facilities at Dingle Oceanworld Aquarium for initial processing within 6 hours. The sampling technique of bagging fronds and holdfast before separating the material on land potentially risks mixing species attached to the frond with those of the holdfast. However, fronds mainly host attached organisms such as hydroids and bryozoans and fewer mobile species (Walls *et al.*, 2017) so this form of contamination is likely to be minimal. The alternative, of cutting fronds in situ, risks dislodging loosely attached species on one treatment (primed ropes), but not the other (unprimed ropes). The mesh bags were untied before randomly pre-selected 10 cm sections (n=3) of each dropper were excised from the 1 m dropper (Figure 2A+B). In the rare event that any randomly selected section of primed dropper was not entirely covered in holdfast structures, an alternative section was chosen. The frond and stipe material of primed samples were cut just above the holdfast and stored in sealed plastic bags containing 100 % ethanol for a separate study. The 10 cm primed and unprimed rope sections were stored in separate sealed plastic bags containing 100 % ethanol. All samples were transported back to the laboratory for further processing.

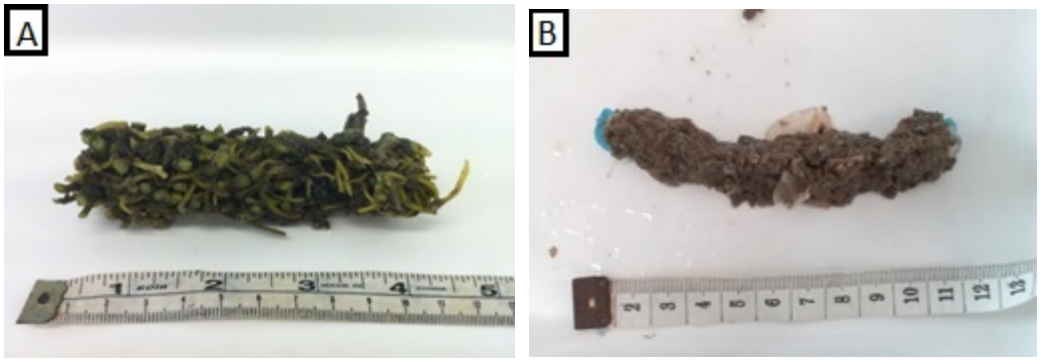


Fig. 2. Morphology of 10 cm section (A) primed *Alaria esculenta* holdfast, (B) unprimed section

## Sample processing

The 10 cm replicate primed *A. esculenta* and unprimed sections were removed from the plastic bag and all material (i.e. kelp holdfasts, epiphytes and fauna) was cleaned from the substratum. Due to the morphology of cultivated kelp holdfasts, individual holdfasts could not be removed as the haptera grow intertwined with each other (Figure 2A, also see Walls *et al.,* (2016) for comparison of morphology of wild and cultivated kelp holdfasts). All collected epibionts from primed and unprimed samples were washed over a 0.5 mm sieve and stored in 100 % ethanol for later identification. All collected flora and fauna were identified to species level where possible (using Hayward, 1988; Hayward & Ryland, 2002; Bunker *et al.*, 2012). Taxonomy was cross-checked using web resources (WoRMS Editorial Board 2016) and samples were stored in 100 % ethanol.

## Statistical analysis

The impact of priming ropes with kelp sporophytes was examined using univariate tests of diversity, multivariate tests of assemblage composition and regressions of mean occurrence days for different treatment and year combinations. To compare functional diversity between primed and unprimed treatments, species were grouped into categories based on morphology (algae): thin filamentous algae, foliose algae and leathery macrophyte (Steneck & Dethier, 1994; Eriksson *et al.*, 2002); and feeding strategies (fauna): suspension feeder, detritivore, carnivore, omnivore or herbivore. Where species spanned these categories, their predominant model of feeding was recorded (Sheppard *et al.*, 1980). Occurrences of functional groups were pooled by treatment/month combinations and tested using a two-way sampling date x treatment model with functional group number as a response variable. Univariate analysis of variance (ANOVA) was used to examine temporal differences in species richness of dropper communities (n = 3 10 cm sections pooled) between primed and

unprimed treatments using the model of month (fixed factor) crossed with treatment (fixed factor) for years 2014 and 2015 separately. Dropper sections were pooled to make droppers rather than 10 cm sections the basic unit of replication. The pooling was carried out as variation within droppers and between sections was not part of the hypothesis of interest.

The differences between community composition from different months and years for the primed and unprimed treatments were compared using multivariate tests. A Simpson's dissimilarity matrix was generated from the presence/absence data for pooled dropper communities from years 2014 and 2015 separately for the 3 primed and 3 unprimed treatments and different months (April, May and June). Simpson's dissimilarity values are 0 when assemblages from separate samples are identical and scaled to 100 when there are no species in common between separate samples. Simpson's dissimilarity has the advantage that it only measures the compositional turnover and is not affected by the relative difference in species richness between samples (Baselga, 2010). This makes dissimilarities measured by Simpson's index easier to interpret than is the case for indices that mix turnover and species richness components of dissimilarity (e.g. Sørensen's index). The PRIMER software used for multivariate analysis does not calculate Simpson's dissimilarities, so these were calculated from species presence/absence data in EXCEL using the PopTools add-on (Hood, 2014) and dissimilarity matrices were subsequently imported into PRIMER. All samples were ordinated using a multidimensional scaling plot (MDS) (Shepard, 1962; Kruskal, 1964a, 1964b) in PRIMER V6®, giving the position of each dropper community (n=3 sections pooled) in two-dimensional space based on its species composition for years 2014 and 2015 separately.

Permutational multivariate analysis of variance (PERMANOVA, Anderson *et al.,* 2008) was used to test for differences in multivariate species assemblages among primed and unprimed communities using the model of month (fixed factor) crossed with treatment (fixed factor) for years 2014 and 2015 separately, based on 9999 unrestricted permutations of raw data. PERMDISP routine revealed that the variation in multivariate dispersion (around the centroid) was not significant ($p > 0.5$) for all factors (month and treatment).

Where significant differences between sampling dates and treatments were detected, a variation on SIMPER analysis was conducted to highlight the species that contributed most to the observed differences. The SIMPER programme in PRIMER could not be used as Simpson's dissimilarity is not compatible with the algorithm used in the programme. Instead, we conducted a SIMPER-like analysis, hereafter referred to as a test of species influence (Walls *et al.*, 2017), by comparing the observed dissimilarity within and between samples when all

species were included to the dissimilarities generated by excluding each species individually. The average between-group and within-group dissimilarities were compared to see whether omitting a species made the groups appear more or less similar. In summary, if a species makes groups more similar when excluded from the matrix, it suggests that the species makes a contribution to the observed differences between groups. Ratios of between to within group dissimilarities were normalized to facilitate comparisons. Any species greater than one standard deviation of the mean normalized score was identified as having an above average contribution to the overall dissimilarity between the groups.

To test the predictability of the presence of species on primed and unprimed droppers from Year 1 (2014) to Year 2 (2015), we used day of year to calculate the central tendency of species occurrence. Day of year is the number assigned to a whole solar day that starts at 1 on the 1$^{st}$ of January and finishes at 365 on 31$^{st}$ of December (non-leap year), e.g., sampling took place on 7$^{th}$ April 2015 which is day of year 97. Central tendency is the average day when a species was observed, weighted by the frequency of occurrence. For example, if a species has high occurrence in the first sampling date, lower occurrence in the middle sampling date and does not occur in the final sampling date then the mean occurrence day will be somewhere between the first and second sampling date. The central tendency method as described by Colebrook (1979) can identify changes in the timing of seasonal cycles (Edwards & Richardson, 2004; Moore *et al.*, 2011). To test if species arrival times were similar between the different treatment droppers within the same year the mean species arrival times were compared between treatments within years 2014 and 2015 separately. Regression analysis was conducted using Minitab v16 to test if arrival times of commonly occurring species were significantly related. A slope close to 1 is expected if the timing of species presences is the same from year to year. If the relationship between occurrence days is significant, but the slope is not close to 1, this implies that the order of species occurrence is similar between sets of samples, but the rate of species arrival varies between years.

## RESULTS

In total, we recorded 81 species inhabiting the primed and unprimed 10 cm dropper sections. 54 species were recorded on 2014 primed sections and 63 species were recorded on the 2015 primed sections. 28 % of all taxa were unique to primed samples including the lumpsucker *Cyclopterus lumpus* Linnaeus (1758), the ascidian *Ciona intestinalis* Linnaeus (1767), and the polychaete *Nereimyra punctata* Müller (1788). Only 6 % of taxa (5 species) were unique to the unprimed ropes, 4 of these were algal species including the kelps *Saccorhiza polyschides* (Lightfoot) Batters (1902) and *Saccharina latissima*, the brown algae *Desmarestia viridis* (O.F. Müller) J.V. Lamourx (1813) and the green algae *Ulva* sp. Linnaeus (1753). The only faunal species unique to unprimed samples was the gastropod *Patella pellucida* Linnaeus (1758) which is usually associated with the kelp fronds on which it exclusively feeds (Hayward, 1988). As only one individual was recorded, the occurrence of this species is not particularly informative. Variation in sample depth along the 1 m dropper ropes or location of the droppers within the farm did not cause any differences in the species richness or community composition of primed and unprimed 10 cm sections. A full list of species sampled on both primed and unprimed samples can be found in Table S1 in supplementary material.

Thin filamentous algae were the most dominant algal functional group for both treatments, with algal diversity higher (4/6 dates) in unprimed treatments when compared to primed treatments in the same month. The suspension feeders were the most common faunal groups for both treatments, followed by omnivores and detritivores (Table 1). A full list of species recorded and their abundance is provided in Supplementary Material (Table S1). Functional group richness increased with time since deployment ($F_{2,6} = 5.7$, $p < 0.05$), but there were no effects of treatment.

302 Table 1. Number of species in each functional group for primed (P) and unprimed (UP) dropper treatments

303 sampled in April, May and June 2014 and 2015.

| Functional Groups | April 2014 P | April 2014 UP | May 2014 P | May 2014 UP | June 2014 P | June 2014 UP | April 2015 P | April 2015 UP | May 2015 P | May 2015 UP | June 2015 P | June 2015 UP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Thin Filamentous Algae** | 1 | 2 | 2 | 3 | 3 | 4 | 4 | 4 | 3 | 4 | 4 | 5 |
| **Foliose Algae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| **Leathery Macrophyte** | 1 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 3 | 1 | 3 |
| **Suspension Feeder** | 5 | 3 | 9 | 9 | 15 | 12 | 8 | 7 | 16 | 13 | 22 | 17 |
| **Detritivore** | 2 | 2 | 6 | 3 | 7 | 4 | 3 | 4 | 8 | 5 | 6 | 7 |
| **Carnivore** | 1 | 1 | 5 | 6 | 6 | 1 | 4 | 1 | 6 | 3 | 7 | 4 |
| **Omnivore** | 7 | 4 | 8 | 8 | 10 | 8 | 6 | 5 | 8 | 7 | 10 | 10 |
| **Herbivore** | 0 | 0 | 1 | 1 | 1 | 2 | 0 | 0 | 1 | 1 | 1 | 1 |
| **Other** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |

304

## 305 Community composition and development of epibionts on primed *Alaria esculenta* 10 306 cm holdfast sections and unprimed sections.

307 Species richness at the seaweed farm site in Ventry increased from time of deployment until the end of the
308 study, for both primed and unprimed treatments. Apart from the accumulation of species with time, the patterns
309 of species richness were not consistent across factors (Treatment 2014 $F_{1,24} = 0.126$, P = 0.725, Treatment 2014
310 x Months 2014 $F_{2,24} = 1.433$, P = 0.258; Treatment 2015 x Months $F_{2,24} = 0.088$, 2015 P = 0.914). Significant
311 differences in species richness were recorded for month for both years (Months 2014 $F_{2,24} = 34.608$, P = <
312 0.001; Months 2015 $F_{2,24} = 91.555$, P = < 0.001), and treatment is significant for 2015 from the ANOVA (Figure
313 3, Treatment 2015 $F_{1,24} = 16.796$, P = < 0.001). Species richness within 2015 was higher in the primed treatment
314 than in the unprimed treatment for each sampling month. For 2014 primed species richness was higher than
315 unprimed species richness for April samples, however, richness was lower for primed samples from May and
316 June than unprimed samples (Figure 3).

317

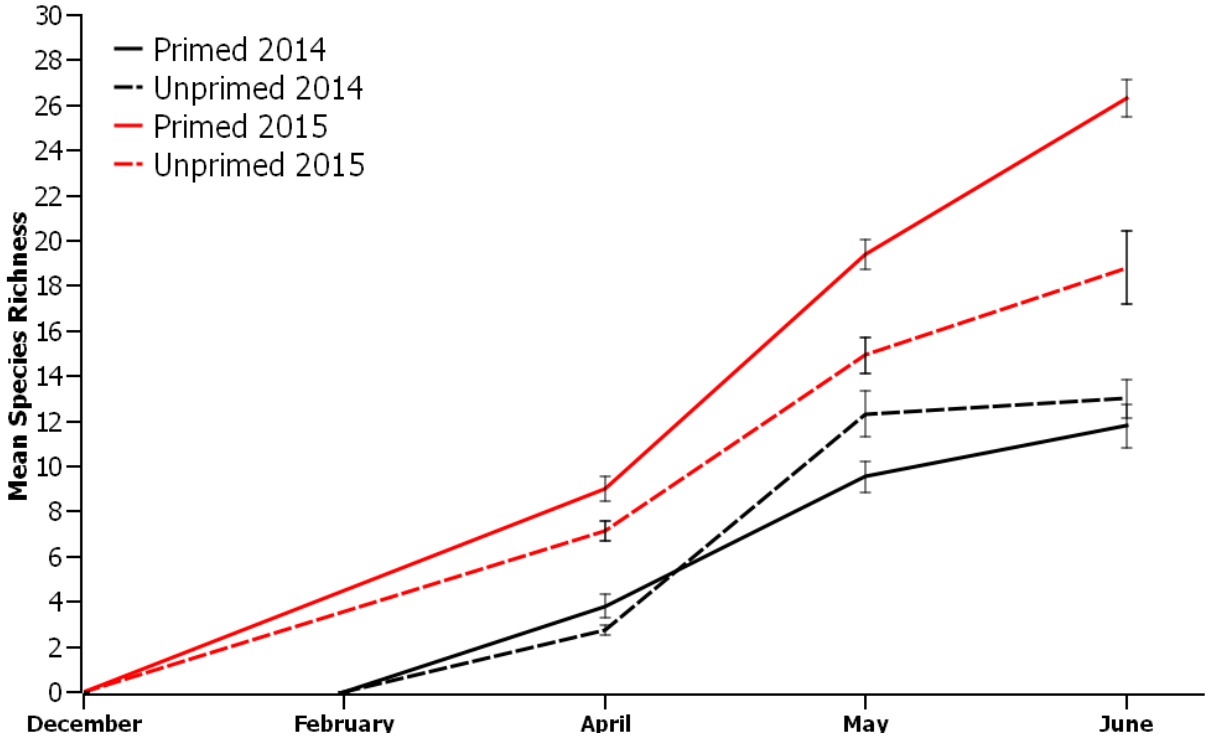


Fig.3. Species richness (mean ± SE) on primed *Alaria esculenta* holdfast sections and unprimed sections sampled in April, May and June 2014 and 2015. Species richness represents the number of taxa identified on 10 cm section (n = 3) from each dropper (n = 5).

Due to the high stress values of the MDS plots for year 2014 (0.22 Figure 4A) and for year 2015 (0.23 Figure 4B) patterns of differences among assemblages from separate months and treatments were difficult to examine. A stress value of > 0.20 indicates the data are only partially represented by the two-dimensional plot and little reliance should be placed on the finer detail of the plot (Clarke & Warwick, 1994). However, the broad-scale pattern shows a separation of early April communities to later May and June communities which show little separation, with month also being significant from the PERMANOVA analysis for both years 2014 and 2015 (Table 2A+B, $p < 0.05$). There is also a separation of community assemblages between primed and unprimed treatments. This pattern is evident from the PERMANOVA analysis with species assemblage composition differing between treatment (primed and unprimed) from the analysis again for both years 2014 and 2015 (Table 2A+B, $p < 0.01$). Interestingly, the month crossed with treatment interaction was significant for year 2015 only (Table 2B, $p < 0.05$). The pairwise tests reveal that this significant interaction is not due to whether treatments are different in one particular month, but the interaction implies that month within a treatment are sometimes different and sometimes not.

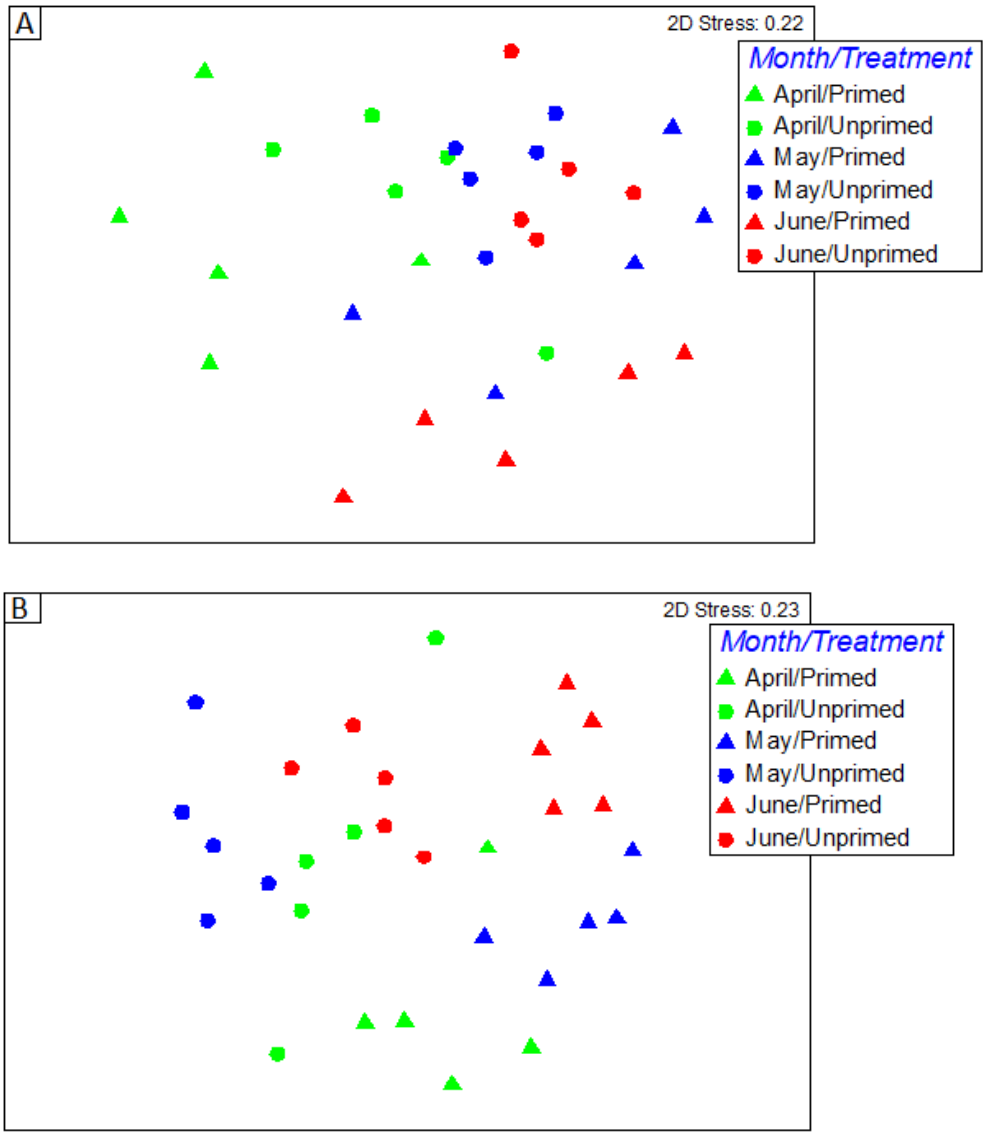


Fig. 4. Two-dimensional multidimensional scaling plot of 30 primed and unprimed dropper samples (n = 3 pooled): 15 from primed treatment and 15 from unprimed treatment, based on presence/absence Simpson's dissimilarity matrix of species collected from each primed and unprimed section. (A) Samples from year 2014 (stress = 0.22). (B) Samples from year 2015 (stress = 0.23).

Table 2: Permutational multivariate analyses of variance based on Simpsons dissimilarity matrix based on presence/ absence data for dropper community (n = 3 10 cm rope sections pooled) sampled over months (April, May and June) on different treatments (primed and unprimed) for (A) year 2104 and (B) year 2015. All tests were conducted using unrestricted permutation of raw data with 9999 permutations.

| **Table 2A: PERMANOVA Year 2014** | | | | | |
|---|---|---|---|---|---|
| **Source** | ***df*** | **SS** | **MS** | ***F*** | **P (perm)** |
| **Month** | 2 | 0.7802 | 0.3901 | 7.3524 | 0.0001** |
| **Treatment** | 1 | 0.32709 | 0.32709 | 6.1649 | 0.0011** |
| **Month x Treatment** | 2 | 0.14437 | $7.2187 \times 10^{-2}$ | 1.3606 | 0.2803 |
| **Residuals** | 24 | 1.2734 | $5.3057 \times 10^{-2}$ | | |
| **Total** | 29 | 2.525 | | | |

| **Table 2B: PERMANOVA Year 2015** | | | | | |
|---|---|---|---|---|---|
| **Source** | ***df*** | **SS** | **MS** | ***F*** | **P (perm)** |
| **Month** | 2 | 0.10493 | $5.2466 \times 10^{-2}$ | 2.1916 | 0.0496* |
| **Treatment** | 1 | 0.39384 | 0.39384 | 16.452 | 0.0001** |
| **Month x Treatment** | 2 | 0.11995 | $5.9977 \times 10^{-2}$ | 2.5054 | 0.0298* |
| **Residuals** | 24 | 0.57454 | $2.3939 \times 10^{-2}$ | | |
| **Total** | 29 | 0.1933 | | | |

*df*: degrees of freedom; SS: sum of squares; MS: mean squares; F-ratio of within-group variation to between-group variation, P (perm) permutational probability value, * $p < 0.05$, ** $p < 0.001$

A test of species influence was conducted to determine which taxa were the major contributors to the observed dissimilarity in assemblage structure between primed and unprimed treatments within the same sampling months (Table 3). Most of the species responsible for dissimilarity between treatments were algae and sessile faunal species. The species with higher occurrence on the primed samples were from a variety of different phyla and dissimilarities were not characterised by any specific group. These included the amphipod *Jassa* fem. Montague (1808) present in April 2014 samples, and the polychaete *Harmothoe* sp. Kinberg (1856) present in May 2014 samples, the bryozoan *Electra pilosa* Lamourx (1816) which contributed to differences between treatments in both June 2014 and May 2015, the amphipod *Gammarellus homari* Herbst (1793) present in April 2015 and the bivalve molluscs *Anomia epipphium* Linnaeus (1758) and *Hiatella arctica* Linnaeus (1767) which were responsible for some of the differences observed in June 2015. The unprimed treatment was generally characterised by a higher occurrence of algal species during each sampling month. The filamentous brown algae sp. and the red alga *Ceramium* sp. Wiggers (1780) were major contributors to the observed differences between

370 treatments and were present in April 2014 (filamentous brown algae sp. only), May 2014 and 2015 and June
371 2015. Laminariales juveniles Migula (1909) were present in higher occurrence in all sampling months except
372 May 2014 when they did not contribute to observed dissimilarities. The red algae *Polysiphonia* sp. Greville
373 (1823) and *Lomentaria clavellosa* Lyngbye (1819) were present in June 2015. The only non-algal species which
374 contributed to the dissimilarity between treatments with higher occurrence in the unprimed treatment was
375 *Harpacticoida* indent. Sars (1903) present in June 2014. From the dissimilarity scores May 2014 and April 2015
376 treatments are less dissimilar than June 2015, however primed and unprimed treatments sampled in June 2014
377 and May 2015 are the most dissimilar (> 2.00 dissimilarity score, Table 3).
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390 Table 3: Test of species influence to determine the species contributing to observed differences in the structure
391 of assemblages between primed and unprimed treatments. Dissimilarity scores are the ratio of average between-
392 group dissimilarities to within-group dissimilarities for each pairwise comparison. Normalized score is the
393 reduction in dissimilarity score when excluding the species of interest, normalized to mean = 0, SD = 1 using
394 the mean and standard deviation of all individual species' scores. A higher loss in dissimilarity indicates that a
395 species is important in distinguishing the dates compared.

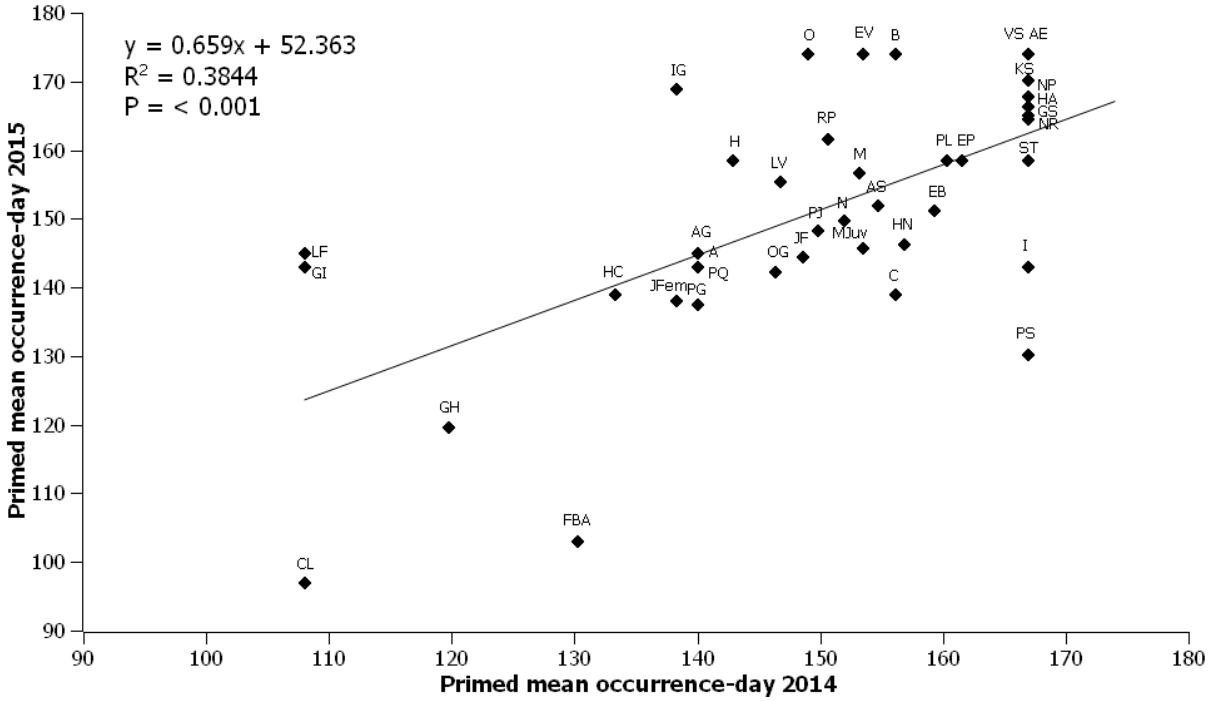
| **Months** | **Dissimilarity score all species included** | **Species responsible for dissimilarity** | **Normalized score** | **Species occurrence (max. 15 mo$^{-1}$)** | | |
|---|---|---|---|---|---|---|
| | | | | **Primed** | | **Unprimed** |
| **April 2014 Primed & Unprimed** | 1.17 | Filamentous brown algae sp. | 2.36 | 7 | < | 10 |
| | | Jassa fem. | 1.05 | 15 | > | 12 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **May 2014** | 1.56 | Filamentous brown algae sp. | 4.25 | 1 | < | 14 |
| **Primed & Unprimed** | | *Ceramium* sp. | 1.72 | 2 | < | 12 |
| | | *Harmothoe* sp. | 1.34 | 6 | > | 2 |
| **June 2014** | 2.00 | *Electra pilosa* | 4.13 | 12 | > | 0 |
| **Primed & Unprimed** | | *Laminariales* juv. | 3.28 | 0 | < | 15 |
| | | *Harpacticoida* indent. | 1.94 | 3 | < | 15 |
| **April 2015** | 1.62 | *Laminariales* juv. | 4.70 | 0 | < | 12 |
| **Primed & Unprimed** | | *Gammarellus homari* | 1.37 | 10 | > | 0 |
| **May 2015** | 2.06 | Filamentous brown algae sp. | 4.42 | 0 | < | 14 |
| **Primed & Unprimed** | | *Laminariales* juv. | 4.37 | 0 | < | 14 |
| | | *Electra pilosa* | 1.19 | 15 | > | 1 |
| | | *Ceramium* sp. | 1.15 | 7 | < | 14 |
| **June 2015** | 1.84 | *Ceramium* sp. | 4.09 | 4 | < | 15 |
| **Primed & Unprimed** | | Filamentous brown algae sp. | 3.07 | 1 | < | 11 |
| | | *Polysiphonia* sp. | 2.64 | 5 | < | 14 |
| | | *Laminariales* juv. | 1.89 | 5 | < | 12 |
| | | *Anomia epipphium* | 1.59 | 15 | > | 1 |
| | | *Hiatella arctica* | 1.10 | 15 | > | 2 |
| | | *Lomentaria clavellosa* | 1.06 | 1 | < | 8 |

## Predictability of holdfast assemblages on primed and unprimed treatments

Shared species between and within treatments included algal species and both sessile and mobile faunal species. The temporal pattern of shared holdfast species was consistent between years for the primed treatment; however, this pattern was not the same in the unprimed treatment. The regression of mean species occurrence in 2014 and 2015 was significant in primed treatments ($p < 0.001$; Figure 5A). In contrast, patterns of mean species arrival on unprimed treatments were not consistent between 2014 and 2015 ($p > 0.05$; Figure 5B).

Recruitment by shared species to both treatments was similar within a year. The timing of species occurrence was consistent across treatments within the same year for both 2014 and 2015. The regression slope relating mean day of year of shared species occurrence samples was significant within both years ($p < 0.05$; Figure S1A & S1B Supplementary Material).

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411

412 Fig. 5A. The relationship between mean day of year occurrences for all shared species in 2014 and 2015 on

413 primed holdfast samples.

414 Legend: CL: *Cyclopterus lumpus*; LF: *Laomedea flexuosa*; GI: *Gammarus insensibilis*; GH: *Gammarellus*

415 *homari*; FBA: filamentous brown algae sp; HC: Harpacticoid Copepods; JFem: *Jassa* sp. female; IG: *Idotea*

416 *granulosa*; PG: Pycnogonida indent.; A: *Aora* sp.; AG: *Aora gracilis*; PQ: *Polycera quadrilineata*; H:

417 *Harmothoe* sp.; OG: *Obelia geniculata*; LV: *Lacuna vincta*; JF: *Jassa falcata*; O: Ostracoda indent.; PJ:

418 *Parajassa pelagica*; RP: *Rissoa parva*; N: Nematoda indent.; M: *Myrianida* sp.; EV: *Eulalia viridis*; MJuv:

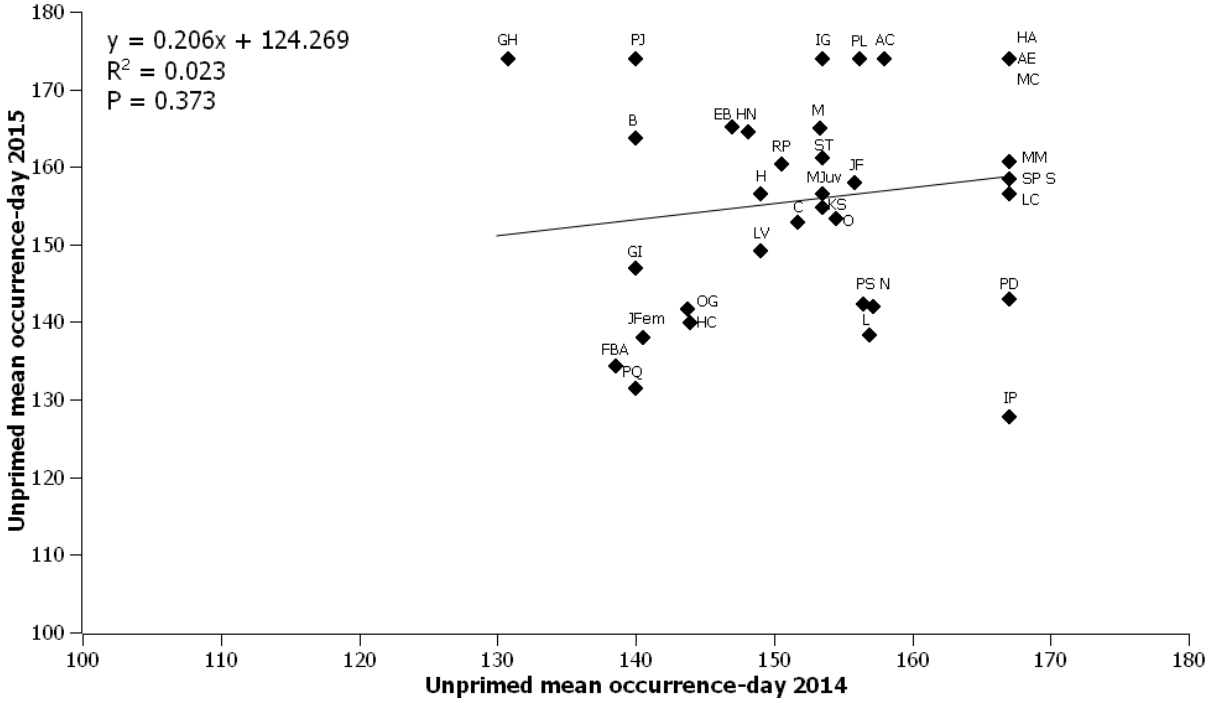
419 *Mytilus* sp. juvenile; AS: *Asterias* sp.; B: *Balanus* sp.; C: *Ceramiales* sp; HN: *Hardametopa nasuta*; EB:

420 *Eusyllis blomstrandi*; PL: *Pisidia longicornis*; EP: *Electra pilosa*; ST: *Spirobranchus triqueter*; NR: *Nereiphylla*

421 *rubiginosa*; NP: *Nereimyra punctate*; GS: *Gitana sarsi*; KS: *Kellia suborbicularis*; HA: *Hiatella arctica*; I:

422 *Idotea* sp.; PS: *Polysiphonia* sp; AE: *Anomia epipphium*; VS: *Vesicularia spinosa*

423




424

425 Fig. 5B. The relationship between mean day of year occurrences for all shared species in 2014 and 2015 on
426 unprimed samples.

427 Legend: GH: *Gammarellus homari*; FBA: filamentous brown algae sp; GI: *Gammarus insensibilis*; B: Balanus
428 sp.; PQ: *Polycera quadrilineata*; .; PJ: *Parajassa pelagica*; JFem: *Jassa* sp. female; OG: *Obelia geniculata*; HC:
429 Harpacticoid Copepods; EB: *Eusyllis blomstrandi*; HN: *Hardametopa nasuta*; LV: *Lacuna vincta*; H:
430 *Harmothoe* sp.; RP: *Rissoa parva*; C: *Ceramiales* sp; M: *Myrianida* sp.; ST: *Spirobranchus triqueter*; IG: *Idotea*
431 *granulosa*; MJuv: *Mytilus* sp. juvenile; KS: *Kellia suborbicularis*; O: Ostracoda indent; JF: *Jassa falcata*; PL:
432 *Pisidia longicornis*; PS: *Polysiphonia* sp; L: *Laminariales* juvenile.; N: Nematoda indent.; AC: *Acari* sp.; SP:
433 *Saccorhiza polyschides*; LC: *Lomentaria clavellosa*; MM: *Membranipora membranacea*; PD: *Platynereis*
434 *dumerilii*; S: Sipuncula indent.; IP: *Idotea pelagica*; MC: *Musculus costulatus*; AE: *Anomia epipphium*; HA:
435 *Hiatella arctica*

# DISCUSSION

## Community composition and development of epibionts on primed *Alaria esculenta* 10 cm holdfast sections and unprimed sections.

Diverse assemblages developed on both primed *Alaria esculenta* sections and unprimed sections. The assemblage developed from unfouled material at deployment to 63 individual taxa sampled on the primed treatment and 54 taxa sampled on the unprimed treatment in 2015 which had higher species richness than 2014. This general build-up of species over sampling months followed a seasonal pattern of development from time of deployment in February (2014) and December (2015) until harvest in June for both growing seasons. The species identified on our primed samples have all been previously recorded on wild or cultivated kelp holdfasts (Jones, 1971; Christie *et al.*, 2003; Blight & Thompson, 2008; Tuya *et al.*, 2011; Schaal *et al.*, 2012; Walls *et al.*, 2016). Although we did not record species abundance directly, the dominant faunal groups based on species occurrence in our primed samples were amphipod crustaceans, polychaetes and molluscs. This agrees with previous studies of wild kelp holdfasts from European waters including Blight & Thompson (2008), Christie *et al.,* (2003) and Walls *et al.,* (2016), the latter of which is the only previous study of cultivated holdfast assemblages to our knowledge.

Functional diversity was dominated by suspension feeders, omnivores and detritivores for both our primed and unprimed samples. However, algal diversity was higher on unprimed samples with 4 of the 5-unique species on unprimed samples being algae, e.g. the kelps *Saccorhiza polyschides* and *Saccharina latissima*, the green algae *Ulva* sp., and *Desmarestia viridis* a filamentous brown alga. Consistently higher species richness in primed samples during early sampling suggests that habitat availability is very important for the colonization of species during early successional stages (April). This importance lessens as communities develop, leading to less consistency in the treatments with higher species richness. The rapid colonisation of this novel habitat suggests that the species have either planktonic larval settlement or if they have direct development, species are highly mobile (Walls *et al.*, 2016). Fouling epibionts such as bryozoans, hydroids, molluscs and crustaceans begin to settle in spring and early summer, which coincides with deployment and the cultivation period for kelps (Walls *et al.*, 2017). The sources of these colonizing species remain unknown. The nearest wild kelp populations are between 250 m (mouth of Ventry Harbour opening up to Dingle Bay) and 1 km (northeast direction from farm in Ventry Harbour) away from the farm site. Cultivation practices are not harmonized within the sector or even

between years at the same site, however at Ventry Harbour over the duration of this study, the header and dropper ropes were taken in from sea and cleaned after each harvest season, also anchor chains are cleaned *in situ* by divers at irregular intervals. Thus, over-wintering of fauna on the farm and re-colonization of the growing kelp and infrastructure in spring is unlikely to occur.

Analysis of the community composition of primed and unprimed treatments revealed several important patterns, which remained constant between years. Communities were distinct between treatments and also between months, with primed samples showing more separation during community development than unprimed samples. The dissimilarity results imply that species are not simply accumulated over time; there are compositional differences between early and later samples. Change in community composition was through addition of new species and replacement of early colonisers. This pattern was more evident in primed samples: with the species filamentous brown algae sp., showing higher occurrence in April samples with much reduced occurrence in later May and June samples.

Algal species were revealed to be the main cause of variation between community composition of treatments from the test of species influence. Filamentous brown algae sp., *Ceramiales* sp., *Laminariales* juveniles, *Polysiphonia* sp. and *Lomentaria clavellosa* were more closely associated with the unprimed treatment. The presence of *A. esculenta* from the beginning of colonisation may pre-empt other algal species from settling and dominating the primed droppers. Benedetti-Cecchi (2000) studied the effect of disturbance on turf and canopy-forming algae in Italy. He found that canopy-forming algae dominated cleared patches of substratum during their main recruitment period; even though turf-forming algae were initially present they were replaced by canopy-forming algae. However, turf-forming algae would characterise early stages of colonisation and mature assemblages in patches that were cleared outside of the main recruitment period of the canopy-forming algae. In our study, unprimed samples were dominated by filamentous and ephemeral algae species, which seemed unable to colonise primed samples potentially because of the presence of *A. esculenta*. Furthermore, the bryozoan *Electra pilosa* was only present on primed samples late in the sampling period with very low occurrence in the unprimed treatment. *E. pilosa* settles in early spring (Ryland & Hayward, 1977) and is found to be out competed by *Membranipora membranacea* Linnaeus (1767) on kelp fronds (Førde *et al.*, 2016; Walls *et al.*, 2017), however because *M. membranacea* is highly selective in habitat (Ryland, 1962) it does not thrive in other habitats thus *E. pilosa* is the dominant bryozoan on these samples. Additionally, in the absence of *M.*

*membranacea*, *E. pilosa* is also selective and was unable to settle on the unprimed samples. The polychaete *Harmothoe* sp., the amphipod *Gammarellus homari*, and the bivalves *Anomia ephippium* and *Hiatella arctica*, were all more closely associated with the primed treatment and have all been previously recorded on kelp holdfasts (Christie *et al.*, 2003; Blight & Thompson, 2008; Walls *et al.*, 2016) and are suggested to utilise the crevices provided by the structurally complex holdfast morphology.

## Predictability of primed and unprimed communities from one year to the next

Between growing seasons, the mean occurrence-days of common species on primed samples were found to be predictable. This consistent pattern for primed samples was significant despite variation in deployment date and sampling date between years and factors such as water temperature, turbidity, irradiance and salinity presumably also varying from one year to the next (Walls *et al.*, 2017). This pattern was not replicated for shared species on unprimed samples between years (Figure 5B). However, the colonisation of shared species between treatments within the same year was shown to be predictable (Figure S1A & S1B in Supplementary Material). This informs us that treatment did not affect arrival and colonisation of shared epibionts within years and suggests that primed *A. esculenta* ropes are habitat to a specific assemblage whereas the unprimed habitat had more loosely associated assemblages. Interestingly, a predictable pattern was also observed between shared frond epibionts on cultivated *A. esculenta* (Walls *et al.*, 2017), but patterns of mobile fauna from the same site sampled at the same time were not predictable (un published data). Walls *et al.,* (2017) suggested that the predictability of organism arrival times observed on their frond samples could be attributed to flushing times within the bay affecting local larval pools; this could also be a probable explanation for our primed holdfast assemblages (Herben, 2005; Jessopp *et al.*, 2007). Ecological priming with juvenile *A. esculenta* sporophytes provides a biological platform that influences the development of predictable communities whereas the unprimed substratum leads to the development of unpredictable communities. This is an important consideration from a management perspective and the ability to understand the timing of occurrence of organisms and predict their arrival has significant benefits for the seaweed cultivation industry and management (Walls *et al*., 2017). With this knowledge, seaweed farmers can exert some control over the quality of their crop by being able to decide on a date-by-site basis when the optimum time to harvest is to avoid detrimental fouling species attaching to their crop. This study was only conducted over a two-year period and analysis of communities over longer durations would be required before definitive conclusions can be made.

Ecological priming using kelp sporophytes provides the complex physical structure that is the holdfast, which has many interstitial spaces for epibionts to colonise. The holdfast also offers protection from predators and adverse environmental conditions (Norderhaug *et al.*, 2002), accumulates food sources (Moore, 1972) and increases the area of substratum and volume of habitable space available for colonisation (Ojeda & Santelices, 1984; Teagle *et al.*, 2017). Cultivated kelp holdfasts likely provide similar resources to colonising communities as wild kelps (Walls *et al.*, 2016). Hauser *et al.,* (2006) experimentally altered the complexity of artificial holdfast mimics and found significantly lower diversity on low complexity mimics in comparison to those with higher complexity. The organisms inhabiting low complexity habitats need to be highly mobile to escape predation as there is less physical structure for refuge and food may be more difficult to find as it is not concentrated within the structure (Hauser *et al.*, 2006). Hauser *et al.,* (2006) also suggests that higher complexity habitats offer a greater surface area for attachment of species, in addition to providing a larger surface area to catch organisms floating in that water column. The latter point is especially interesting in the context of our droppers which are suspended within the water column, and thus are more likely to attract larvae and pelagic organisms drifting in the water. As a consequence of ecological priming our primed samples are more predictable than unprimed samples potentially due to the foundational structure provided by kelp holdfasts.

## Succession of epibiont assemblages of primed *Alaria esculenta* holdfasts

There appear to be no published descriptions of succession on cultivated holdfasts so comparisons must be drawn from wild holdfast studies and successional studies from alternative systems. Kelp successional studies used holdfast volume rather than holdfast age to analyse succession, due to difficulties in determining the age of holdfasts partly because of the indistinct nature of growth rings and the lack of comparative data using age rather than volume in other studies (Smith *et al.*, 1996). Interestingly, several studies suggest that successional processes do not involve species replacement but rather an additive progression (Ojeda & Santelices, 1984; Smith *et al.*, 1996; Smith, 2000; Teagle *et al.*, 2017). Smith *et al.,* (1996) found that while early colonists on *Ecklonia radiata* (Agardh) Agardh (1848) holdfasts generally had a shift in dominance in larger holdfasts, all species that were recorded in smaller holdfasts were also present in larger samples. This was evident in *Macrocystis pyrifera* (Linnaeus) C. Agardh (1820) holdfasts that had a shift in dominance of polychaetes in smaller samples to a more diverse community in larger samples in which echinoids and decapod crustaceans were dominant (Ojeda & Santelices, 1984). Ojeda & Santelices (1984) suggested that this form of succession

555 may be more characteristic of habitats that grow, such as corals and sponges. This type of successional process
556 is dissimilar to many other habitats where succession has been studied, including our cultivated kelp holdfasts,
557 in which community change involved the replacement of early colonists with later species (Connell & Slatyer,
558 1977; Dean & Connell, 1987; Platt & Connell, 2003; Cifuentes *et al.*, 2010). In rocky shore and artificial
559 habitats, the timing of disturbance or the creation of free-space can influence richness and abundance of initial
560 colonizers which in turn affects succession, (Sousa, 1979; Dayton *et al.*, 1984; Benedetti-Cecchi & Cinelli,
561 1993; Underwood & Chapman, 2006; Cifuentes *et al.*, 2010; Valdivia *et al.,* 2014). This is partly due to
562 seasonality in organisms' reproductive patterns and/or growth and seasonal variation in environmental
563 conditions (Jenkins & Martins, 2010). This effect of timing was observed in the initial differences between early
564 primed samples. However, as clearly evident from our primed samples and a number of other successional
565 studies, varying successional trajectories subsequently converge towards a local climax community (Underwood
566 & Chapman, 2006; Cifuentes *et al.*, 2010; Antoniadou, 2014; Evans *et al.*, 2016; Walls *et al.*, 2017). One such
567 study, Cifuentes *et al.,* (2010), proposed that initial and intermediate successional stages can be highly variable,
568 while late stages are highly deterministic if a dominant species is present that uses the available energy
569 efficiently. This leads to a convergence of communities with different start points. As our study followed
570 succession over the first 4-7 months of development of primed and unprimed communities we do not know if
571 these communities will converge into one climax state dominated by a superior competitor(s), either within the
572 individual treatments or between treatments. However, from previously conducted studies it is highly probable
573 that they could converge to similar end-point communities.
574

575 ## Outlook

576 This study identifies a unique habitat provided by the ecological priming of droppers with *A. esculenta*
577 sporophytes, creating a complex physical structure with a distinct community when compared to unprimed
578 ropes. This distinct community may be attracted to the habitat and refuge provided by the interstitial spaces
579 between the holdfast haptera and its ability to accumulate food. The effect of primed ropes may reflect
580 suppression of algal species that would otherwise colonize suspended ropes, and the facilitation of species that
581 have a particular association with kelps. The restoration of kelp forests (Carney *et al.*, 2005; Yu *et al.*, 2012;
582 Marzinelli *et al.,* 2016) and the transplantation of habitat forming species (Perkol-Finkel *et al.*, 2012; Ferrario *et*
583 *al.*, 2016; Strain *et al.,* 2017) onto artificial structures have gained increased interest recently with attempts to
584 mitigate the potential negative anthropogenic impacts of ocean sprawl (Airoldi & Beck, 2007; Firth *et al.*,

2016a, 2016b; Strain *et al.,* 2017). Rope has even been used as a method for enhancing productivity and biodiversity enhancement on pier pilings (Paalvast et al., 2012), and its physical structure mimicked on pre-cast concrete habitat enhancement units (Perkol-Finkel & Sella, 2015). Deployment of seaweed lines may aid in habitat restoration by supplying spores and gametophytes to wild kelp beds that have been damaged by anthropogenic impacts, or by transplanting seeded kelp juveniles directly onto artificial structures (Marzinelli *et al.,* 2009). Ecologically priming the substratum with kelp seems likely to lead to the development of particular predictable associated communities. Colonisation onto kelps can occur from settlement of larvae or migration by mobile fauna (Walls *et al.*, 2016, 2017). The duration of the 'seeding' effect remains to be defined. Communities may become more similar over time (depending on successional processes and dominant species), or the influence of a kelp-dominated habitat may increase the longer the longlines are left in the water column. The cultivation practices for kelps are subject to change and development. Harvesting practices may be adjusted so that holdfasts, stipes and small fronds remain in place for more than one growing season, however the applicability of these techniques depends on culture species. Another area for future research is the impact of primed ropes on primary and secondary productivity associated with these communities. An assessment of productivity could increase the importance of primed communities through quantification of the ecosystem services they provide (Beaumont *et al.,* 2007). The importance of priming effects may depend on the case-by-case details of cultivation practice, and there is a need for further research to fully understand the novelty of habitats created by seaweed cultivation.

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615 REFERENCES

616 **Airoldi, L. & Beck, M.W.** (2007) Loss, status and trends for coastal marine habitats of Europe. *Oceanography*
617 *and Marine Biology: An Annual Review*, 35, 345–405.
618 **Anderson, M.J., Gorley, R.N. & Clarke, K.R.** (2008) *PERMANOVA+ for PRIMER: guide to software and*
619 *statistical methods. PRIMER-E, Plymouth, UK*.
620 **Antoniadou, C. (2014)** Succession patterns of polychaetes on algal-dominated rocky cliffs (Aegean Sea,
621 Eastern Mediterranean). *Marine Ecology*, 35, 281–291.
622 **Arroyo, N.L., Maldonado, M., Pérez-Portela, R. & Benito, J.** (2004) Distribution patterns of meiofauna
623 associated with a sublittoral *Laminaria* bed in the Cantabrian Sea (north-eastern Atlantic). *Journal of*
624 *Marine Biology*, 144, 231–242.
625 **Baselga, A.** (2010) Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and*
626 *Biogeography*, 19, 134–143.
627 **Beaumont, N.J., Austen, M.C., Atkins, J.P., Burdon, D., Degraer, S., Dentinho, T.P., Derous, S., Holm, P.,**
628 **Horton, T., van Ierland, E., Marboe, A.H., Starkey, D.J., Townsend, M. & Zarzycki, T.** (2007).
629 Identification, definition and quantification of goods and services provided by marine biodiversity:
630 Implications for the ecosystem approach. *Marine Pollution Bulletin* 54, 253-265.
631 **Benedetti-Cecchi, L.** (2000) Predicting direct and indirect interactions during succession in a mid-littoral rocky
632 shore assemblage. *Ecological Monographs*, 70, 45–72.
633 **Benedetti-Cecchi, L. & Cinelli, F.** (1993) Early patterns of algal succession in a midlittoral community of the
634 Mediterranean sea: a multifactorial experiment. *Journal of Experimental Marine Biology and Ecology*,
635 169, 15–31.
636 **Bishop M.J., Mayer-Pinto M., Airoldi L., Firth L.B., Morris R.L., Loke L.H.L., Hawkins S.J., Naylor**
637 **L.A., Coleman R.A., Chee S.Y., Dafforn K.A.** (2017) Effects of ocean sprawl on ecological
638 connectivity: impacts and solutions. *Journal of Experimental Marine Biology and Ecology*, 492, 7–30
639 **Blight, A.J. & Thompson, R.C.** (2008) Epibiont species richness varies between holdfasts of a northern and a
640 southerly distributed kelp species. *Journal of the Marine Biological Association of the United Kingdom*,
641 88, 469–475.
642 **Bunker, F.S.D., Maggs, C.A., Brodie, J.A. & Bunker, A.R.** (2012) *Seaweeds of Britian and Ireland*. Wild
643 Nature Press, Plymouth, UK.
644 **Carney, L.T., Waaland, J.R., Klinger, T. & Ewing, K.** (2005) Restoration of the bull kelp *Nereocystis*
645 *luetkeana* in nearshore rocky habitats. *Marine Ecology Progress Series*, 302, 49–61.
646 **Chapman, M.G. & Underwood, A.J.** (2011) Evaluation of ecological engineering of "armoured" shorelines to
647 improve their value as habitat. *Journal of Experimental Marine Biology and Ecology*, 400, 302–313.
648 **Christie, H., Jørgensen, N.M. & Norderhaug, K.M.** (2007) Bushy or smooth, high or low; importance of
649 habitat architecture and vertical position for distribution of fauna on kelp. *Journal of Sea Research*, 58,
650 198–208.
651 **Christie, H., Jørgensen, N.M., Norderhaug, K.M. & Waage-Nielsen, E.** (2003) Species distribution and
652 habitat exploitation of fauna associated with kelp (*Laminaria digitata*) along the Norwegian coast.
653 *Journal of Marine Biological Association of the United Kingdom*, 83, 687–699.
654 **Cifuentes, M., Krueger, I., Dumont, C.P., Lenz, M. & Thiel, M.** (2010) Does primary colonization or
655 community structure determine the succession of fouling communities? *Journal of Experimental Marine*
656 *Biology and Ecology*, 395, 10–20.
657 **Clarke, K.R. & Warwick, R.M.** (1994) *Changes in marine communities: an approach to statistical analysis*
658 *and interpretation*. *PRIMER-E Ltd*. 2nd edn. Primer-E, Plymouth.
659 **Colebrook, J.M.** (1979) Continuous plankton records: Seasonal cycles of phytoplankton and copepods in the
660 North Atlantic Ocean and the North Sea. *Marine Biology*, 51, 23–32.
661 **Connell, J.H. & Slatyer, R.O.** (1977) Mechanisms of succession in natural communities and their role in
662 community stability and organisation. *The American Naturalist*, 111, 1119–1144.
663 **Dafforn, K.A., Glasby, T.M., Airoldi, L., Rivero, N.K., Mayer-Pinto, M. & Johnston, E.L**. (2015) Marine
664 urbanization: An ecological framework for designing multifunctional artificial structures. *Frontiers in*
665 *Ecology and the Environment*, 13, 82–90.
666 **Dayton, P.K., Currie, V., Gerrodette, T., Keller, B.D., Rosenthal, R., & Tresca, D.V.,** (1984) Patch
667 dynamics and stability of some California kelp communities. *Ecological Monographs*, 54, 253–289.
668 **Dean, R.L. & Connell, J.H.** (1987) Marine invertebrates in an algal succession. I. Variations in abundance and
669 diversity with succession. *Journal of Experimental Marine Biology and Ecology*, 109, 195–215.
670 **Duarte, C.M., Pitt, K.A., Lucas, C.H., Purcell, J.E., Uye, S.-I., Robinson, K., Brotz, L., Decker, M.B.,**

**Sutherland, K.R., Malej, A., Madin, L., Mianzan, H., Gili, J.-M., Fuentes, V., Atienza, D., Pagés, F., Breitburg, D., Malek, J., Graham, W.M. & Condon, R.H.** (2012) Is global ocean sprawl a cause of jelly fish blooms? *Frontiers in Ecology and the Environment* 11, 91–97.

**Edwards, M. & Richardson, A.J.** (2004) Impacts of climate change on marine pelagic phenology and trophic mismatch. *Nature*, 430, 881–884.

**Edwards, M. & Watson, L.** (2011) *Cultivating* Laminaria digitata *Aquaculture Explained No. 26 Bord Iscaigh Mhara*. Dublin.

**Eriksson, B.K., Johansson, G. & Snoeijs, P.** (2002) Long-term changes in the macroalgal vegetation of the inner Gullmar Fjord, Swedish Skagerrak coast. *Journal of Phycology*, 38, 284–296.

**Evans, A.J., Firth, L.B., Hawkins, S.J., Morris, E.S., Goudge, H. & Moore, P.J.** (2016) Drill-cored rock pools: An effective method of ecological enhancement on artificial structures. *Marine and Freshwater Research*, 67, 123–130.

**FAO.** (2016) *The state of world fisheries and aquaculture 2016*. ROME.

**Ferrario, F., Iveša, L., Jaklin, A., Perkol-Finkel, S. & Airoldi, L.** (2016) The overlooked role of biotic factors in controlling the ecological performance of artificial marine habitats. *Journal of Applied Ecology*, 53, 16–24.

**Firth, L.B., Knights, A.M., Bridger, D., Evans, A.J., Mieszkowska, N., Moore, P.J., O'Connor, N.E., Sheehan, E.V., Thompson, R.C. & Hawkins, S.J.** (2016a) Ocean Sprawl: Challenges and opportunities for biodiversity management in a changing world. *Oceanography and Marine Biology: An Annual Review*, 54, 189–262.

**Firth, L.B., Browne, K.A., Knights, A.M., Hawkins, S.J. & Nash, R.** (2016b) Eco-engineered rock pools: a concrete solution to biodiversity loss and urban sprawl in the marine environment. *Environmental Research Letters*, 11, 94015.

**Firth, L.B. & Hawkins, S.J.** (2011) Introductory comments - Global change in marine ecosystems: Patterns, processes and interactions with regional and local scale impacts. *Journal of Experimental Marine Biology and Ecology*, 400, 1–6.

**Firth, L.B., Thompson, R.C., White, F.J., Schofield, M., Skov, M.W., Hoggart, S.P.G., Jackson, J., Knights, A.M. & Hawkins, S.J.** (2013) The importance of water-retaining features for biodiversity on artificial intertidal coastal defence structures. *Diversity and Distributions*, 19, 1275–1283.

**Førde, H., Forbord, S., Handå, A., Fossberg, J., Ariff, J., Johnsen, G. & Reitan, K.I**. (2016) Development of bryozoan fouling on cultivated kelp (*Saccharina latissim*) in Norway. *Journal of Applied Phycology*, 28, 1225–1234.

**Guiry, M.D.** (1989) *Uses and cultivation of seaweed. Camera di Commercio Industria Artigiantoe Agricoltura; Università Degli Studi.*

**Hauser, A., Attrill, M.J. & Cotton, P.A.** (2006) Effects of habitat complexity on the diversity and abundance of macrofauna colonising artificial kelp holdfasts. *Marine Ecology Progress Series*, 325, 93–100.

**Hayward, P.J.** (1988) *Animals on Seaweed. Great Britain*. The Richmond Publishing Co. Ltd., Surrey.

**Hayward, P.J. & Ryland, J.S.** (2002) *Handbook of the marine fauna of North-West Europe:* Oxford University Press, Oxford.

**Heery, E.C., Bishop, M.J., Critchley, L.P., Bugnot, A.B., Airoldi, L., Mayer-Pinto, M., Sheehan, E.V., Coleman, R.A., Loke, L.H., Johnston, E.L. & Komyakova, V.** (2017) Identifying the consequences of ocean sprawl for sedimentary habitats. *Journal of Experimental Marine Biology and Ecology*, 492, 31-48.

**Herben, T.** (2005) Species pool size and invasibility of island communities: A null model of sampling effects. *Ecology Letters*, 8, 909–917.

**Hood, G.M.** (2014) PopTools version 3.2.5. Available on the internet. www.poptools.org [accessed on 24 May 2016].

**Jenkins, S. & Martins, G.** (2010) Succession on hard substrata In: Biofouling. In (ed. by Dürr, S. & Thomason, J.). Wiley-Blackwell, West Sussex.

**Jessopp, M., Mulholland, O.R., McAllen, R., Johnson, M.P., Crowe, T.P. & Allcock, A.L.** (2007) Coastline configuration as a determinant of structure in larval assemblages. *Marine Ecology Progress Series*, 352, 67–75.

**Jones, D.J.** (1971) Ecological studies on macroinvertebrate populations associated with polluted kelp forests in the North Sea. *Helgoländer Wissenschaftliche Meeresuntersuchungen*, 22, 417–441.

**Jones, D.J.** (1972) Changes in the ecological balance of invertebrate communities in kelp holdfast habitats of some polluted North Sea waters. *Helgoländer Wissenschaftliche Meeresuntersuchungen*, 23, 417–441.

**Kain, J.M.** (1963) Aspects of the biology of *Lamlnaria hyperborea*. II. Age. Weight. Length. *Journal of Marine Biological Association of the United Kingdom*, 129–151.

**Kruskal, J.B.** (1964a) Nonmetric multidimensional scaling: A numerical method. *Psychometrika*, 29, 115–129.

**Kruskal, J.B.** (1964b) Multidimensional scaling by optimizing goodness-of-fit to a nonmetric hypothesis. *Psychometrika*, 29, 115–129.

**Marzinelli, E.M., Zagal, C.J., Chapman, M.G. & Underwood, A.J.** (2009) Do modified habitats have direct or indirect effects on epifauna? *Ecology,* 90, 2948–2955.

**Marzinelli, E.M., Leong, M.R., Campbell, A.H., Steinberg, P.D. & Vergés, A.** (2016) Does restoration of a habitat- forming seaweed restore associated faunal diversity?. *Restoration Ecology*, 24(1), 81-90.

**Moore, P.** (1972) Particulate matter in the sublittoral zone of an exposed coast and its ecological significance with special reference to the fauna inhabiting kelp holdfasts. *Journal of Experimental Marine Biology and Ecology*, 10, 59–80.

**Moore, P.** (1973) The kelp fauna of northeast Britain. I. Introduction and the physical environment. *Journal of Experimental Marine Biology and Ecology*, 13, 97–125.

**Moore, P.J., Thompson, R.C. & Hawkins, S.J.** (2011) Phenological changes in intertidal con-specific gastropods in response to climate warming. *Global Change Biology*, 17, 709–719.

**Norderhaug, K.M.** (2004) Use of red algae as hosts by kelp-associated amphipods. *Marine Biology*, 144, 225–230.

**Norderhaug, K.M., Christie, H. & Rinde, E**. (2002) Colonisation of kelp imitations by epiphyte and holdfast fauna; a study of mobility patterns. *Journal of Marine Biology*, 141, 965–973.

**Ojeda, F.P. & Santelices, B.** (1984) Invertebrate communities in holdfasts of the kelp *Macrocystis pyrifera* from southern Chile. *Marine Ecology Progress Series*, 16, 65–73.

**Paalvast, P., van Wesenbeeck, B.K., van der Velde, G. & de Vries, M.B.,** 2012. Pole and pontoon hulas: an effective way of ecological engineering to increase productivity and biodiversity in the hard-substrate environment of the port of Rotterdam. *Ecological engineering*, 44, 199-209.

**Park, T.S., Rho, Y.G., Gong, Y.G. & Lee, D.Y.** (1990) A harpacticoid copepod parasitic in the cultivated brown alga *Undaria pinnatifida* in Korea. *Bulletin of the Korean Fisheries Society*, 23, 439–442.

**Perkol-Finkel, S. & Sella, I.** (2015) Harnessing urban coastal infrastructure for ecological enhancement. *Proceedings of the Institution of Civil Engineers*, 168(3) 102-110.

**Perkol-Finkel, S., Ferrario, F., Nicotera, V. & Airoldi, L**. (2012) Conservation challenges in urban seascapes: Promoting the growth of threatened species on coastal infrastructures. *Journal of Applied Ecology*, 49, 1457–1466.

**Peteiro, C. & Freire***, Ó.* (2013) Epiphytism on blades of the edible kelps *Undaria pinnatifida* and *Saccharina latissima* farmed under different abiotic conditions. *Journal of the World Aquaculture Society*, 44, 706–715.

**Platt, W.J. & Connell, J.H.** (2003) Natural disturbances and directional replacement of species. *Ecological Monographs*, 73, 507–522.

**Ryland, J.** (1962) The association between polyzoa and algal substrata. *Journal of Animal Ecology*, 31, 331–338.

**Ryland, J.S. & Hayward, P.J.** (1977) *British anascan bryozoans*. Academic Press, London.

**Scarratt, D.J.** (1961) *The fauna of Laminaria holdfasts. PhD thesis, Univerity of Wales, Aberystwyth*.

**Schaal, G., Riera, P. & Leroux, C.** (2009) Trophic significance of the kelp *Laminaria digitata*, (Lamour.) for the associated food web: a between-sites comparison. *Estuarine, Coastal and Shelf Science*, 85, 565–572.

**Schaal, G., Riera, P. & Leroux, C**. (2012) Food web structure within kelp holdfasts (*Laminaria*): a stable isotope study. *Journal of Marine Ecology*, 33, 370–376.

**Schultze, K., Janke, K., Krüß, A. & Weidemann, W.** (1990) The macrofauna and macroflora associated with *Laminaria digitata* and *L. hyperborea* at the island of Helgoland (German Bight, North Sea). *Helgoländer Meeresuntersuchungen*, 44, 39–51.

**Shepard, R**. (1962) The analysis of proximities: Multidimensional scaling with an unknown distance function. II. *Psychometrika*, 27, 219–246.

**Sheppard, C.R.C., Bellamy, D.J. & Sheppard, A.L.S.** (1980) Study of the fauna inhabiting the holdfasts of *Laminaria hyperborea* (gunn.) fosl. along some environmental and geographical gradients. *Marine Environmental Research*, 4, 25–51.

**Smale, D.A., Burrows, M.T., Moore, P., O'Connor, N. & Hawkins, S.J.** (2013) Threats and knowledge gaps for ecosystem services provided by kelp forests: a northeast Atlantic perspective. *Ecology and Evolution*, 3, 4016–4038.

**Smith, S.D.A.** (1996) The macrofaunal community of *Ecklonia radiata* holdfasts: Variation associated with sediment regime, sponge cover and depth. *Australian Journal of Ecology*, 21, 144–153.

**Smith, S.D.A**. (2000) Evaluating stress in rocky shore and shallow reef habitats using the macrofauna of kelp holdfasts. *Journal of Aquatic Ecosystem Stress and Recovery*, 7, 259–272.

**Smith, S.D.A., Simpson, R.D. & Cairns, S.C.** (1996) The macrofaunal community of *Ecklonia radiata* holdfasts: description of the faunal assemblage and variation associated with differences in holdfast volume. *Australian Journal of Ecology*, 21, 81–95.

**Sousa, W.P.** (1979) Experimental Investigations of Disturbance and Ecological Succession in a Rocky Intertidal Algal Community. *Ecological Monographs*.

**Steneck, R.S. & Dethier, M.N.** (1994) A Functional Group Approach to the Structure of Algal-Dominated Communities. *Oikos*, 69, 476.

**Strain, E.M.A., Morris, R.L., Coleman, R.A., Figueira, W.F., Steinberg, P.D., Johnston, E.L. & Bishop, M.J.** (2017). Increasing microhabitat complexity on seawalls can reduce fish predation on native oysters. *Ecological Engineering*, 7(22) 9567-9579.

**Strain, E.M.A., Olabarria, C., Mayer- Pinto, M., Cumbo, V., Morris, R.L., Bugnot, A.B., Dafforn, K.A., Heery, E., Firth, L.B., Brooks, P. & Bishop, M.J.,** (2017) Eco- engineering urban infrastructure for marine and coastal biodiversity: which interventions have the greatest ecological benefit? *Journal of Applied Ecology*, 55, 426–441.

**Teagle, H., Hawkins, S.J., Moore, P.J. & Smale, D.A.** (2017) The role of kelp species as biogenic habitat formers in coastal marine ecosystems. *Journal of Experimental Marine Biology and Ecology,* 492, 81-98.

**Tett, P.** (1990) A three layer vertical and microbiological process model for shelf seas. Birkenhead, *Proudman Oceanographic Laboratory*, 85pp. (Proudman Oceanographic Laboratory, Report no. 14)

**Thiel, M. & Vásquez, J.** (2000) Are kelp holdfasts islands on the ocean floor?—indication for temporarily closed aggregations of peracarid crustaceans. *Hydrobiologia*, 440, 45–54.

**Tuya, F., Larsen, K. & Platt, V.** (2011) Patterns of abundance and assemblage structure of epifauna inhabiting two morphologically different kelp holdfasts. *Hydrobiologia*, 658, 373–382.

**Underwood, A.J. & Chapman, M.G.** (2006) Early development of subtidal macrofaunal assemblages: Relationships to period and timing of colonization. *Journal of Experimental Marine Biology and Ecology*, 330, 221–233.

**Valdivia, N., Buschbaum, C., Thiel, M.,** (2014) Succession in intertidal mussel bed assemblages on different shores: species mobility matters. *Marine Ecology Progress Series,* 497, 131-142.

**Walls, A.M., Edwards, M.D., Firth, L.B. & Johnson, M.P.** (2017) Successional changes of epibiont fouling communities of the cultivated kelp *Alaria esculenta*: predictability and influences. *Aquaculture Environment Interactions*, 9, 55–69.

**Walls, A.M., Kennedy, R., Fitzgerald, R.D., Blight, A.J., Johnson, M.P. & Edwards, M.D.** (2016) Potential novel habitat created by holdfasts from cultivated *Laminaria digitata* : assessing the macroinvertebrate assemblages. *Aquaculture Environment Interactions*, 8, 157–169.

**WoRMS Editorial Board.** (2016) World register of marine species. www.marinespecies.org [accessed on 15 April 2016].

**Yu, Y.Q., Zhang, Q.S., Tang, Y.Z., Zhang, S.B., Lu, Z.C., Chu, S.H. & Tang, X.X**. (2012) Establishment of intertidal seaweed beds of *Sargassum thunbergii* through habitat creation and germling seeding. *Ecological Engineering*, 44, 10–17.